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(54) Title: TANGO-71, TANGO-73, TANGO-74, TANGO-76, AND TANGO-83 NUCLEIC ACID MOLECULES AND POLYPEPTIDES (57) Abstract The invention relates to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83, and uses thereof.		

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TANGO-71, TANGO-73, TANGO-74, TANGO-76, AND TANGO-83
NUCLEIC ACID MOLECULES AND POLYPEPTIDES

Cross Reference to Related Applications

This application claims priority from U.S. Serial
5 Number 60/054,966, filed August 6, 1997 and U.S. Serial
Number 60/058,108, filed September 5, 1997.

Summary of the Invention

The invention relates to the discovery and
characterization of Tango-71, Tango-73, Tango-74, Tango-
10 76, and Tango-83. Tango-71 is a human protein which is
approximately 90% identical to murine ADAMTS-1. Tango-73
is a human protein that is 48% identical to rat RVP.1
(Briehl et al., *Mol. Endocrinol.* 5:1381, 1991). Tango-74
is a human protein with homology to TRAIL receptor (Pan
15 et al., *Science* 276:111, 1997). Tango-76 is a rat
protein which is approximately 40% identical to murine
ADAMTS-1. Tango-83 is expressed by stimulated human
astrocytes.

The invention features isolated nucleic acid
20 molecules encoding Tango-71, Tango-73, Tango-74, Tango-
76, or Tango-83 polypeptides; isolated nucleic acid
molecules encoding polypeptides which are substantially
similar to Tango-71, Tango-73, Tango-74, Tango-76, or
Tango-83; and isolated nucleic acid molecules which
25 hybridize under stringent conditions to a nucleic acid
molecule having the sequence of the protein coding
portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID
NO:7, or SEQ ID NO:9.

The invention also features a host cell which
30 includes an isolated nucleic acid molecule encoding
Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 and a
nucleic acid vector (e.g., an expression vector; a vector
which includes a regulatory element; a vector which
includes a regulatory element selected from the group
35 consisting of the cytomegalovirus hCMV immediate early

- 2 -

gene, the early promoter of SV40 adenovirus, the late promoter of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors; vector which includes a regulatory element which directs tissue-specific expression; a vector which includes a reporter gene; a vector which includes a reporter gene selected from the group selected from the group consisting of β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r , G418 r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT); a vector that is a plasmid, a vector that is a virus; and a vector that is a retrovirus) containing an isolated nucleic acid molecule encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention also features substantially pure Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides; a substantially pure polypeptide which includes a first portion and a second portion, the first portion including a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide and the second portion including a detectable marker.

- 3 -

The invention also features an antibody that selectively binds to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., a monoclonal antibody).

5 The invention also features a pharmaceutical composition which includes a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide.

The invention includes methods for diagnosing a disorder associated with aberrant expression of a protein
10 of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83), the method including obtaining a biological sample from a patient and measuring the expression of the protein in the biological sample, wherein increased or decreased expression of the protein
15 in the biological sample compared to a control indicates that the patient suffers from a disorder associated with aberrant expression of the protein.

The invention encompasses isolated nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-
20 76, or Tango-83 or a polypeptide fragment thereof; vectors containing these nucleic acid molecules; cells harboring recombinant DNA encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; fusion proteins which include all or a portion of Tango-71, Tango-73, Tango-74,
25 Tango-76, or Tango-83; transgenic animals which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; and recombinant knock-out animals which fail to express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention encompasses nucleic acids that have
30 a sequence that is substantially identical to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 nucleic acid sequence. A nucleic acid molecule which is substantially identical to a given reference nucleic acid molecule is hereby defined as a nucleic acid molecule having a
35 sequence that has at least 85%, preferably 90%, and more

- 4 -

preferably 95%, 98%, 99% or more identity to the sequence of the given reference nucleic acid molecule.

The invention also includes polypeptides which are substantially identical to Tango-71, Tango-73, Tango-74, 5 Tango-76, or Tango-83 (e.g., polypeptides that are substantially identical to the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10).

A polypeptide which is "substantially identical" 10 to a given reference polypeptide molecule is a polypeptide having an amino acid sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the amino acid sequence of the given reference polypeptide.

15 To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second 20 amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in 25 the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., 30 overlapping positions) x 100). Preferably, the two sequences are the same length.

The determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a 35 mathematical algorithm utilized for the comparison of two

- 5 -

sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules. Id. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

- 6 -

The nucleic acid molecules of the invention can be inserted into vectors, as described below, which will facilitate expression of the insert. The nucleic acid molecules and the polypeptides they encode can be used directly as diagnostic or therapeutic agents, or (in the case of a polypeptide) can be used to generate antibodies that, in turn, are therapeutically useful. Accordingly, expression vectors containing the nucleic acid molecules of the invention, cells transfected with these vectors, the polypeptides expressed, and antibodies generated, against either the entire polypeptide or an antigenic fragment thereof, are among the preferred embodiments.

A transformed cell is any cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid encoding a polypeptide of the invention (e.g., a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide).

An isolated nucleic acid molecule is a nucleic acid molecule that is separated from the 5' and 3' coding sequences with which it is immediately contiguous in the naturally occurring genome of an organism. Isolated nucleic acid molecules include nucleic acid molecule which are not naturally occurring, e.g., nucleic acid molecules created by recombinant DNA techniques.

Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand.

The invention also encompasses nucleic acid molecules that hybridize, preferably under stringent conditions, to a nucleic acid molecule encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., a nucleic acid molecule having the sequence shown in SEQ ID NO:1, 3, 5, 7, or 9). Preferably the

- 7 -

hybridizing nucleic acid molecule consists of 400, more preferably 200 nucleotides. Preferred hybridizing nucleic acid molecules have a biological activity possessed by Tango-71, Tango-73, Tango-74, Tango-76, or
5 Tango-83.

The invention also features substantially pure or isolated Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides, including those that correspond to various functional domains of Tango-71, Tango-73, Tango-
10 74, Tango-76, or Tango-83, or fragments thereof.

The polypeptides of the invention can be produced recombinantly, chemically synthesized, or purified from tissues in which they are naturally expressed, according to standard biochemical methods of purification.

Also included in the invention are functional
15 polypeptides, which possess one or more of the biological functions or activities of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. These functions include the ability to bind some or all of the proteins which
20 normally bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. A functional polypeptide is also considered within the scope of the invention if it serves as an antigen for production of antibodies that specifically
bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-
25 83. In many cases, functional polypeptides retain one or more domains present in the naturally-occurring form of the polypeptide.

The functional polypeptides may contain a primary amino acid sequence that has been modified from those
30 disclosed herein. Preferably these modifications consist of conservative amino acid substitutions, as described herein.

The terms "protein" and "polypeptide" are used herein to describe any chain of amino acids, regardless
35 of length or post-translational modification (for

- 8 -

example, glycosylation or phosphorylation). Thus, the term "Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides" includes full-length, naturally occurring Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein, as well a recombinantly or synthetically produced polypeptide that correspond to a full-length naturally occurring Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or to particular domains or portions of a naturally occurring protein. The term also encompasses mature Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 which has an added amino-terminal methionine (useful for expression in prokaryotic cells).

The term "purified" as used herein refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

Polypeptides or other compounds of interest are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The invention also features antibodies, e.g., monoclonal, polyclonal, and engineered antibodies, which specifically bind Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. An antibody which specifically binds to a given antigen is an antibody that recognizes and binds to a particular antigen, but which does not substantially recognize or bind to other molecules in a sample, e.g., a

- 9 -

biological sample, which includes Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention also features antagonists and agonists of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 that inhibit one or more of the biological activities of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Suitable antagonists can include small molecules (i.e., molecules with a molecular weight below about 500), large molecules (i.e., molecules with a molecular weight above about 500), neutralizing antibodies, polypeptides which compete with a native form of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 for binding to a protein, and nucleic acid molecules that interfere with transcription of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (for example, antisense nucleic acid molecules and ribozymes). Agonists of Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 also include small and large molecules, and antibodies other than neutralizing antibodies.

The invention also features molecules which can increase or decrease the expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (e.g., by influencing transcription or translation). Small molecules (i.e., molecules with a molecular weight below about 500), large molecules (i.e., molecules with a molecular weight above about 500), and nucleic acid molecules that can be used to inhibit the expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (for example, antisense and ribozyme molecules) or to enhance their expression (for example, molecules that bind to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcription regulatory sequences and increase Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcription).

In addition, the invention features substantially pure polypeptides that functionally interact with Tango-

- 10 -

71, Tango-73, Tango-74, Tango-76, or Tango-83, and the nucleic acid molecules that encode them.

The invention encompasses methods for treating disorders associated with aberrant expression or activity
5 of a protein of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83). Thus, the invention includes methods for treating disorders associated with excessive expression or activity of a protein of the invention. Such methods entail administering a compound
10 which decreases the expression or activity of the protein. The invention also includes methods for treating disorders associated with insufficient expression or activity of a protein of the invention. These methods entail administering a compound which
15 increases the expression or activity of the protein.

The invention also features methods for detecting a protein of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83). Such methods include: obtaining a biological sample; contacting the sample with
20 an antibody that specifically binds the protein under conditions which permit specific binding; and detecting any antibody-protein complexes formed.

In addition, the present invention encompasses methods and compositions for the diagnostic evaluation,
25 typing, and prognosis of disorders associated with inappropriate expression or activity of a protein of the invention. For example, the nucleic acid molecules of the invention can be used as diagnostic hybridization probes to detect, for example, inappropriate expression
30 of a protein of the invention or mutations in the gene encoding a protein of the invention gene. Such methods may be used to classify cells by the level of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 expression.

The invention encompasses methods for diagnosing a
35 disorder associated with aberrant activity of a protein

- 11 -

of the invention, the methods including obtaining a biological sample from a patient and measuring the activity of the protein in the biological sample, wherein increased or decreased activity in the biological sample
5 compared to a control indicates that the patient suffers from a disorder associated with aberrant activity of the protein.

The nucleic acid molecules of the invention can be used as primers for diagnostic PCR analysis for the
10 identification of gene mutations, allelic variations and regulatory defects in the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene. The present invention further provides for diagnostic kits for the practice of such methods.

15 The invention features methods of identifying compounds that modulate the expression or activity of a protein of the invention by assessing the expression or activity of the protein in the presence and absence of a selected compound. A difference in the level of
20 expression or activity of the protein indicates that the selected compound is capable of modulating expression or activity of the protein. Expression can be assessed either at the level of gene expression (e.g., by measuring mRNA) or protein expression by techniques that
25 are well known to skilled artisans. The activity of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be assessed functionally.

The preferred methods and materials are described below in examples which are meant to illustrate, not
30 limit, the invention. Skilled artisans will recognize methods and materials that are similar or equivalent to those described herein, and that can be used in the practice or testing of the present invention.

Unless otherwise defined, all technical and
35 scientific terms used herein have the same meaning as

- 12 -

commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of Tango-71.

Figure 2 Nucleotide acid sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of Tango-73.

Figure 3 Nucleotide acid sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) of Tango-74. The ATG encoding the first Met is boxed as is the ATC encoding the final Ile.

Figure 4 Nucleotide acid sequence of a 3' non-coding portion of Tango-74 (SEQ ID NO:11).

Figure 5 Alignment of a portion of the amino acid sequence of Tango-74 (SEQ ID NO:6) and the amino acid sequence of TRAIL.

Figure 6 Partial nucleotide sequence (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) of Tango-76.

- 13 -

Figure 7 Nucleotide sequence of a 5' portion of Tango-83 (SEQ ID NO:9).

Figure 8 Nucleotide sequence of a 3' portion of Tango-83 (SEQ ID NO:10).

5 Figure 9 Alignment of amino acid sequence of Tango-71 and the amino acid sequence of ADAMTS-1.

Figure 10 Alignment of the amino acid sequence of Tango-73 and the amino acid sequence of RVPI.

10 Figure 11 Alignment of the amino acid sequence of Tango-73 and TMVCF.

Figure 12 Northern blot analysis of Tango-73 mRNA.

Figure 13 Northern blot analysis of Tango-83 mRNA.

Figure 14 Alignment of amino acid sequence of Tango-76 and ADAMTS-1.

15 Detailed Description

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Nucleic Acid Molecules

The Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 nucleic acid molecules of the invention can be
20 cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (i.e., either a sense or an antisense strand). Fragments of these molecules are also considered within the scope of the invention, and can be produced, for example, by the polymerase chain
25 reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by in vitro transcription.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that
30 differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. In addition, these nucleic acid molecules are not limited to sequences that only encode

- 14 -

polypeptides, and thus, can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass fragments that are not found as such in the natural state. Thus, the invention encompasses recombinant molecules, such as those in which a nucleic acid molecule (for example, an isolated nucleic acid molecule encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

In the event the nucleic acid molecules of the invention encode or act as antisense molecules, they can be used for example, to regulate translation of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. Techniques associated with detection or regulation of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 expression are well known to skilled artisans and can be used to diagnose and/or treat disorders associated with aberrant Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 expression.

The invention also encompasses nucleic acid molecules that hybridize under stringent conditions to a

- 15 -

nucleic acid molecule encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., nucleic acid molecules having the sequence of the protein coding portion of SEQ ID NO:1, 3, 5, 7, or 9). The cDNA sequences described herein can be used to identify these hybridizing nucleic acids, which include, for example, nucleic acids that encode homologous polypeptides in other species and splice variants of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene in humans or other mammals. Accordingly, the invention features methods of detecting and isolating these nucleic acid molecules. Using these methods, a sample (for example, a nucleic acid library, such as a cDNA or genomic library) is contacted (or "screened") with a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-specific probe (for example, a fragment of SEQ ID NO:1, 3, 5, 7, or 9 that is at least 25 or 50 or 100 nucleotides long). The probe will selectively hybridize to nucleic acids encoding related polypeptides (or to complementary sequences thereof). The probe, which can contain at least 25 (for example, 25, 50, 100, or 200 nucleotides) can be produced using any of several standard methods (see, for example, Ausubel et al., "Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-specific nucleic acid sequence that can be used as a probe to screen a nucleic acid library and thereby detect nucleic acid molecules (within the library) that hybridize to the probe.

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence

- 16 -

that is the reverse and complement of the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the "double
5 helix"). Complete complementarity between the hybridizing regions is not required in order for a duplex to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

10 Typically, hybridization conditions are of low to moderate stringency. These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After
15 hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

20 As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the
25 lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of
30 the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration
35 can be lowered and the temperature increased. Additional

- 17 -

parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized,
5 the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of
10 each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional
15 consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt
20 solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of
25 low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing
30 for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

A second set of conditions that are considered
35 "stringent conditions" are those in which hybridization

- 18 -

is carried out at 50°C in Church buffer (7% SDS, 0.5% NaHPO₄, 1 M EDTA, 1% BSA) and washing is carried out at 50°C in 2X SSC.

Once detected, the nucleic acid molecules can be isolated by any of a number of standard techniques (see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual," 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

The invention also encompasses: (a) expression vectors that contain any of the foregoing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83-related coding sequences operatively associated with a regulatory element (examples of which are given below) that directs the expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide, nucleic acid sequences that are unrelated to nucleic acid sequences encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83, such as molecules encoding a reporter or marker; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention in the host cell.

Recombinant nucleic acid molecules can contain a sequence encoding a soluble Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide; mature Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; or Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 having a signal sequence. A full length Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide; a domain of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; or a fragment thereof may be fused to additional polypeptides, as

- 19 -

described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 or a form that encodes a polypeptide which facilitates secretion. In
5 the latter instance, the polypeptide is typically referred to as a proprotein, which can be converted into an active form by removal of the signal sequence, for example, within the host cell. Proproteins can be converted into the active form of the protein by removal
10 of the inactivating sequence.

The regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or
15 otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major
20 operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

25 Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT),
30 adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As
35 with many of the standard procedures associated with the

- 20 -

practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include
5 a first portion and a second portion; the first portion being a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

10 The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression
15 vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding
20 Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression
25 vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 nucleotide sequences; or mammalian cell
30 systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for

- 21 -

example, the adenovirus late promoter and the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions containing Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides or for raising antibodies to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791, 1983), in which the coding sequence of the insert may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, *Nucleic Acids Res.* 13:3101-3109, 1985; Van Heeke and Schuster, *J. Biol. Chem.* 264:5503-5509, 1989); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhydrosis virus (AcNPV) can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence of the insert may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus

- 22 -

and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (for example, see Smith et al., *J. Virol.* 46:584, 1983; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid molecule of the invention may be ligated to an adenovirus transcription/translation control complex, for example, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (for example, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene product in infected hosts (for example, see Logan and Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659, 1984). Specific initiation signals may also be required for efficient translation of inserted nucleic acid molecules. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the

- 23 -

initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:516-544, 1987).

10 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (for example, glycosylation) and processing (for example, 15 cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems 20 can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the 25 gene product may be used. The mammalian cell types listed above are among those that could serve as suitable host cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For 30 example, cell lines which stably express a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 sequences described above may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA 35 controlled by appropriate expression control elements

- 24 -

(for example, promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 5 1-2 days in an enriched media, and then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can 10 be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that 15 affect the endogenous activity of the gene product.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc.* 20 *Natl. Acad. Sci. USA* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817, 1980) genes can be employed in tk⁻, hgp⁺ or ap⁺ cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: 25 dhfr, which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072, 1981); neo, 30 which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.* 150:1, 1981); and hyg⁺, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147, 1984).

- 25 -

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 nucleic acid molecules are useful in genetic mapping and chromosome identification.

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-

5 83 Polypeptides

The Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides described herein are those encoded by any of the nucleic acid molecules described above and include Tango-71, Tango-73, Tango-74, Tango-76, and
10 Tango-83 fragments, mutants, truncated forms, and fusion proteins. These polypeptides can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene
15 products or compounds that can modulate the activity or expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83, and as pharmaceutical reagents useful for the treatment of disorders associated with aberrant expression or activity of Tango-71, Tango-73, Tango-74,
20 Tango-76, or Tango-83.

Preferred polypeptides are substantially pure Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides, including those that correspond to the polypeptide with an intact signal sequence, the secreted
25 form of a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide. Especially preferred are polypeptides that are soluble under normal physiological conditions.

The invention also encompasses polypeptides that
30 are functionally equivalent to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83. These polypeptides are equivalent to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 in that they are capable of carrying out one or more of the functions of Tango-71, Tango-73, Tango-74,
35 Tango-76, and Tango-83 in a biological system. Preferred

- 26 -

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides have 20%, 40%, 50%, 75%, 80%, or even 90% of one or more of the biological activities of the full-length, mature human form of Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83. Such comparisons are generally based on an assay of biological activity in which equal concentrations of the polypeptides are used and compared. The comparison can also be based on the amount of the polypeptide required to reach 50% of the maximal stimulation obtainable.

Functionally equivalent proteins can be those, for example, that contain additional or substituted amino acid residues. Substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Amino acids that are typically considered to provide a conservative substitution for one another are specified in the summary of the invention.

Polypeptides that are functionally equivalent to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be made using random mutagenesis techniques well known to those skilled in the art. It is more likely, however, that such polypeptides will be generated by site-directed mutagenesis (again using techniques well known to those skilled in the art). These polypeptides may have increased functionality or decreased functionality.

To design functionally equivalent polypeptides, it is useful to distinguish between conserved positions and variable positions. This can be done by aligning the amino acid sequence of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 with the amino acid sequence of the homologous protein from another species. Skilled artisans will recognize that conserved amino acid residues are more likely to be necessary for preservation

- 27 -

of function. Thus, it is preferable that conserved residues are not altered.

Mutations within the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 coding sequence can be made to generate variant Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 genes that are better suited for expression in a selected host cell. For example, N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences which occur, and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified tripeptide sequence (see, for example, Miyajima et al., *EMBO J.* 5:1193, 1986).

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (*Proc. Natl. Acad. Sci. USA* 88: 8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} -nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The polypeptides of the invention can be expressed fused to another polypeptide, for example, a marker

- 28 -

polypeptide or fusion partner. For example, the polypeptide can be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein or a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

The polypeptides of the invention can be chemically synthesized (for example, see Creighton, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., NY, 1983), or, perhaps more advantageously, produced by recombinant DNA technology as described herein. For additional guidance, skilled artisans may consult Ausubel et al. (*supra*), Sambrook et al. ("Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989), and, particularly for examples of chemical synthesis Gait, M.J. Ed. ("Oligonucleotide Synthesis," IRL Press, Oxford, 1984).

The invention also features polypeptides that interact with Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (and the genes that encode them) and thereby alter the function of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Interacting polypeptides can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," which detects protein interactions *in vivo* (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Transgenic animals

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides can also be expressed in transgenic animals. These animals represent a model system for the study of disorders that are caused by or exacerbated by overexpression or underexpression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83, and for the development

- 29 -

of therapeutic agents that modulate the expression or activity of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

Transgenic animals can be farm animals (pigs, goats, sheep, cows, horses, rabbits, and the like) rodents (such as rats, guinea pigs, and mice), non-human primates (for example, baboons, monkeys, and chimpanzees), and domestic animals (for example, dogs and cats). Transgenic mice are especially preferred.

Any technique known in the art can be used to introduce a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148, 1985); gene targeting into embryonic stem cells (Thompson et al., *Cell* 56:313, 1989); and electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803, 1983).

The present invention provides for transgenic animals that carry a the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene in all their cells, as well as animals that carry a transgene in some, but not all of their cells. That is, the invention provides for mosaic animals. The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene can also be selectively introduced into and activated in a particular cell type (Lasko et al., *Proc. Natl. Acad. Sci. USA* 89:6232, 1992). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

- 30 -

When it is desired that the Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be used, vectors containing some nucleotide sequences homologous to an endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene also can be selectively introduced into a particular cell type, thus inactivating the endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene in only that cell type (Gu et al., *Science* 265:103, 1984). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. Once transgenic animals have been generated, expression of the recombinant Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to determine whether integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene-expressing tissue can also be evaluated immunocytochemically using antibodies specific for the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene product.

For a review of techniques that can be used to generate and assess transgenic animals, skilled artisans

- 31 -

can consult Gordon (*Intl. Rev. Cytol.* 115:171-229, 1989), and may obtain additional guidance from, for example: Hogan et al. "Manipulating the Mouse Embryo" (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1986; Krimpenfort et al., *Bio/Technology* 9:86, 1991; Palmiter et al., *Cell* 41:343, 1985; Kraemer et al., "Genetic Manipulation of the Early Mammalian Embryo," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1985; Hammer et al., *Nature* 315:680, 1985; Purcel et al., *Science*, 244:1281, 1986; Wagner et al., U.S. Patent No. 5,175,385; and Krimpenfort et al., U.S. Patent No. 5,175,384 (the latter two publications are hereby incorporated by reference).

Anti-Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Antibodies

Human Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides (or immunogenic fragments or analogs) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, "Solid Phase Peptide Synthesis," *supra*; Ausubel et al., *supra*). In general, the peptides can be coupled to a carrier protein, such as KLH, as described in Ausubel et al., *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or polypeptide. Host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet

- 32 -

hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are
5 contained in the sera of the immunized animals.

Antibodies within the invention therefore include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, and
10 molecules produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides described above and standard
15 hybridoma technology (see, for example, Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel et al., *supra*).

20 In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al., *Nature* 256:495, 1975, and U.S. Patent No. 4,376,110; the human
25 B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be
30 of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this a particularly useful method of
35 production.

- 33 -

Once produced, polyclonal or monoclonal antibodies are tested for specific Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 recognition by Western blot or immunoprecipitation analysis by standard methods, e.g.,
5 as described in Ausubel et al., *supra*. Antibodies that specifically recognize and bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 are useful. For example, such antibodies can be used in an immunoassay to monitor the level of Tango-71, Tango-73, Tango-74, Tango-76, or
10 Tango-83 produced by a mammal (for example, to determine the amount or subcellular location of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83).

Preferably, antibodies of the invention are produced using fragments of the Tango-71, Tango-73,
15 Tango-74, Tango-76, or Tango-83 protein which lie outside highly conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR, and are then
20 cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., *supra*.

In some cases it may be desirable to minimize the
25 potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at
30 least three booster injections.

Antisera is also checked for its ability to immunoprecipitate recombinant Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

- 34 -

The antibodies can be used, for example, in the detection of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 in a biological sample as part of a diagnostic assay. Antibodies also can be used in a screening assay to measure the effect of a candidate compound on expression or localization of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Additionally, antibodies can be used in conjunction with the gene therapy techniques described to, for example, evaluate the normal and/or engineered Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-expressing cells prior to their introduction into the patient. Such antibodies additionally can be used in a method for inhibiting abnormal Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 activity.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851, 1984; Neuberger et al., *Nature*, 312:604, 1984; Takeda et al., *Nature*, 314:452, 1984) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain antibodies against a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

- 35 -

Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to F(ab')₂ fragments that can be produced by pepsin digestion of the antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science*, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can, in turn, be used to generate anti-idiotypic antibodies that resemble a portion of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 using techniques well known to those skilled in the art (see, e.g., Greenspan et al., *FASEB J.* 7:437, 1993; Nissinoff, *J. Immunol.* 147:2429, 1991). For example, antibodies that bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 and competitively inhibit the binding of a binding partner of the protein can be used to generate anti-idiotypes that resemble a binding partner binding domain of the protein and, therefore, bind and neutralize a binding partner of the protein. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens.

Antibodies can be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals are also features of the invention (Green et al., *Nature Genetics* 7:13-21, 1994; see also U.S. Patents 5,545,806 and 5,569,825, both of which are hereby incorporated by reference).

- 36 -

The methods described herein in which anti-Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 antibodies are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 antibody reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of the disorders described below.

10 Antisense Nucleic Acids

Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. These oligonucleotides bind to the complementary Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work

- 37 -

most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, Nature 5 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of the mRNA, could be used in an antisense approach to inhibit translation of endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. Oligonucleotides 10 complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the 15 invention. Whether designed to hybridize to the 5', 3', or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the 20 oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to 25 quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that 30 these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is 35 preferred that the control oligonucleotide is of

- 38 -

approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target
5 sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or
10 phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (as
15 described, e.g., in Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or
20 hybridization-triggered cleavage agents (see, for example, Krol et al., *BioTechniques* 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.* 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide,
25 hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil,
30 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-
35 galactosylqueosine, inosine, N6-isopentenyladenine,

- 39 -

1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
2-methyladenine, 2-methylguanine, 3-methylcytosine,
5-methylcytosine, N6-adenine, 7-methylguanine,
5-methylaminomethyluracil, 5-methoxyaminomethyl-
5 2-thiouracil, beta-D-mannosylqueosine,
5'-methoxycarboxymethyluracil, 5-methoxyuracil,
2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic
acid (v), wybutoxosine, pseudouracil, queosine,
2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-
10 thiouracil, 5-methyluracil, uracil-5-oxyacetic acid
methylester, uracil-5-oxyacetic acid (v), 5-methyl-
2-thiouracil, 2-(3-amino-3-N-2-carboxypropyl) uracil,
(acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at
15 least one modified sugar moiety selected from the group
including, but not limited to, arabinose,
2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense
oligonucleotide comprises at least one modified phosphate
20 backbone selected from the group consisting of a
phosphorothioate, a phosphorodithioate, a
phosphoramidothioate, a phosphoramidate, a
phosphordiamidate, a methylphosphonate, an alkyl
phosphotriester, and a formacetal, or an analog of any of
25 these backbones.

In yet another embodiment, the antisense
oligonucleotide is an α -anomeric oligonucleotide. An
 α -anomeric oligonucleotide forms specific double-stranded
hybrids with complementary RNA in which, contrary to the
30 usual β -units, the strands run parallel to each other
(Gautier et al., *Nucl. Acids. Res.* 15:6625, 1987). The
oligonucleotide is a 2'-O-methylribonucleotide (Inoue
et al., *Nucl. Acids Res.* 15:6131, 1987), or a chimeric
RNA-DNA analog (Inoue et al., *FEBS Lett.* 215:327, 1987).

- 40 -

Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209, 1988), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc. Natl. Acad. Sci. USA* 85:7448, 1988).

The antisense molecules should be delivered to cells that express the protein of interest *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong *pol* III or *pol* II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcripts and thereby prevent translation. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or

- 41 -

become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Bernoist et al., *Nature* 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797, 1988); the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39, 1988).

Ribozymes

Ribozyme molecules designed to catalytically cleave Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA transcripts also can be used to prevent translation and expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (see, e.g., PCT Publication WO 90/11364; Saraver et al., *Science* 247:1222, 1990). While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art (Haseloff et al., *Nature*

- 42 -

334:585, 1988). There are numerous examples of potential hammerhead ribozyme cleavage sites within the nucleotide sequence of human Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 cDNA. Preferably, the ribozyme is
5 engineered so that the cleavage recognition site is located near the 5' end of the mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also
10 include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena Thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug et al., *Science* 224:574,
15 1984; Zaug et al., *Science*, 231:470, 1986; Zug et al., *Nature* 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., *Cell* 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage
20 of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

As in the antisense approach, the ribozymes can be
25 composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme
30 under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a

- 43 -

lower intracellular concentration is required for efficiency.

Other Methods for Reducing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Expression

5 Endogenous Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 gene expression can also be reduced by inactivating the endogenous gene or its promoter using targeted homologous recombination (see, e.g., U.S. Patent No. 5,464,764). For example, a mutant, non-functional
10 Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions) can be used, with or without a selectable marker and/or a negative selectable
15 marker, to transfect cells that express the endogenous gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene. Such approaches are particularly
20 suited for use in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83. However, this approach can be adapted for use in humans, provided
25 the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

 Alternatively, endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene expression can be
30 reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (*i.e.*, the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells in the body (*Helene Anticancer Drug Res.*

- 44 -

6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.* 660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

Detecting Proteins Associated with Tango-71,
Tango-73, Tango-74, Tango-76, or Tango-83

5 The invention also features polypeptides which
interact with Tango-71, Tango-73, Tango-74, Tango-76, or
Tango-83. Any method suitable for detecting protein-
protein interactions may be employed for identifying
transmembrane proteins, intracellular, or extracellular
10 proteins that interact with Tango-71, Tango-73, Tango-74,
Tango-76, or Tango-83. Among the traditional methods
which may be employed are co-immunoprecipitation,
crosslinking and co-purification through gradients or
chromatographic columns of cell lysates or proteins
15 obtained from cell lysates and the use of Tango-71,
Tango-73, Tango-74, Tango-76, or Tango-83 to identify
proteins in the lysate that interact with Tango-71,
Tango-73, Tango-74, Tango-76, or Tango-83. For these
assays, the Tango-71, Tango-73, Tango-74, Tango-76, or
20 Tango-83 polypeptide can be: a full length Tango-71,
Tango-73, Tango-74, Tango-76, or Tango-83; a soluble
extracellular domain of Tango-71, Tango-73, Tango-74,
Tango-76, or Tango-83; or some other suitable Tango-71,
Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide.
25 Once isolated, such an interacting protein can be
identified and cloned and then used, in conjunction with
standard techniques, to identify proteins with which it
interacts. For example, at least a portion of the amino
acid sequence of a protein which interacts with the
30 Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can
be ascertained using techniques well known to those of
skill in the art, such as via the Edman degradation
technique. The amino acid sequence obtained may be used
as a guide for the generation of oligonucleotide mixtures
35 that can be used to screen for gene sequences encoding

- 45 -

the interacting protein. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known (Ausubel, *supra*; and
5 "PCR Protocols: A Guide to Methods and Applications," Innis et al., eds. Academic Press, Inc., NY, 1990).

Additionally, methods may be employed which result directly in the identification of genes which encode proteins which interact with Tango-71, Tango-73, Tango-
10 74, Tango-76, or Tango-83. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of λ gt11 libraries, using labeled Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide or a Tango-
15 71, Tango-73, Tango-74, Tango-76, and Tango-83 fusion protein, e.g., a Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptide or domain fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

20 There are also methods which are capable of detecting protein interaction. A method which detects protein interactions *in vivo* is the two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available
25 from Clontech (Palo Alto, CA).

Compounds which bind Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83

Compounds which bind Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be identified using any
30 standard binding assay. For example, candidate compounds can be bound to a solid support. Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 is then exposed to the immobilized compound and binding is measured (European Patent Application 84/03564).

35 EXAMPLES

- 46 -

Tango-71 cDNA (Fig. 1; SEQ ID NO:1) was isolated from human melanocytes as follows.

Human melanocytes (Clonetics Corporation; San Diego, CA) were expanded in culture with Melanocyte Growth Media (MGM; Clonetics) according to the recommendations of the supplier. When the cells reached ~80-90% confluence, they were starved in MGM without growth factors for 46 hours. The starved cells were then stimulated with complete MGM supplemented with 20 ng/ml TNF (Gibco BRL; Gaithersburg, MD) and cycloheximide (CHI; 40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen; Chatsworth, CA), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL). Complementary DNA was directionally cloned into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to construct a plasmid library. Transformants were picked and grown up for single-pass sequencing. Additionally, astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library.

Northern blot analysis of Tango-71 expression was performed using Tango-71 labeled with ³²P-dCTP using the Prime-It kit (Stratagene, LaJolla, CA). Human mRNA blots (MTNI and MTNII; Clonetech; Palo Alto, CA) were probed and washed at high stringency as recommended by the manufacturer. Tango-71 is expressed as an approximately 6.0 kb transcript in all tissues: heart brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testes, ovary, small intestine, colon, PBLs.

- 47 -

The amino acid sequence of a portion of Tango-71 is 90% identical to the amino acid sequence of murine ADAMTS-1 (FIG. 9), a cellular disintegrin and metalloprotease that is thought to be involved in inflammation and development of cancer cachexia (Kuno et al., *J. Biol. Chem.* 272:556, 1997). Based on sequence comparison to ADAMTS-1, Tango-71, using the amino acid numbering in Figure 9, has the following domains: amino acids 1-160 (metalloproteinase domain, partial); amino acids 170-242 (disintegrin domain); amino acids 257-307 (thrombospondin domain). A less apparent thrombospondin domain is present at amino acid 558-608. Portions of Tango-71 shown in Figure 1, but not in Figure 9, may also be homologous ADAMTS-1. Tango-71 may represent the human homolog of ADAMTS-1 or a splice variant thereof.

Tango-71 expression may be androgen regulated. Tango-71 expression in LNCaP cells, an androgen-dependent prostate cancer cell line, is induced by R1881, a testosterone analog. Tango-71 expression is downregulated in LNCaP cells treated with casodex, an anti-androgen.

Tango-73 cDNA (Fig. 2; SEQ ID NO:2) was isolated from human prostate epithelial cells as follows.

Human prostate epithelial cells (Clonetics) were expanded in culture with Prostate Epithelial Growth Medium (PEGM) (Clonetics). When cells reached confluence cells were grown in Prostate Basal Media (Clonetics) for 24 hours. They were stimulated with PEGM (prostate epithelial growth medium; Clonetics) and 40 ug/ml cycloheximide for 3 hours.

Total RNA was isolated using the RNeasy Midi Kit (Qiagen). Poly (A)+ was isolated using the Oligotex beads (Qiagen). Next, cDNA was constructed using the Superscript-cDNA Synthesis Kit (Gibco BRL). The cDNA was cloned into the expression vector pMET7 using the Sali

- 48 -

and NotI sites in the polylinker. Transformants were picked and sequenced.

Northern blot analysis of Tango-73 expression was carried out as described above. This analysis revealed the presence of 4.0 kb and 3.0 kb transcripts in the placenta and liver. A 4.0 kb transcript was present in lung, kidney, thymus, prostate, spleen, testes, and colon, with the highest expression in lung, pancreas, prostate, and testes.

The amino acid sequence of Tango-73 is 48% identical to rat RVP.1 (Briehl et al., *Mol. Endocrinol.* 5:1381, 1991) and 46.1% identical to TMVCF (Sirotkin et al., *Genomics* 42:245, 1997).

RVP.1 is up-regulated during apoptosis (Briehl et al., *supra*). TMVCF, a 219 amino acid protein with two putative membrane spanning domains, is deleted in velocardio-facial syndrome (Sirotkin et al., *supra*).

Tango-83 (Figs. 7 and 8) and Tango-74 cDNAs (Fig. 5) were isolated from human astrocytes as follows.

Human astrocytes (Clonetics) were expanded in culture with Astrocyte Growth Media (AGM; Clonetics) according to the recommendations of the supplier. When the cells reached ~80-90% confluence, they were stimulated with 200 units/ml Interleukin 1-Beta (Boehringer Mannheim) and cycloheximide (CHI: 40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL). Complementary DNA was directionally cloned into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to construct a plasmid library. Transformants were picked and grown up for single-pass sequencing. Additionally,

- 49 -

astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library.

Northern blot analysis of Tango-83 expression, performed as described above, revealed that Tango-83 is expressed as an approximately 9.0 kb transcript in brain (FIG. 13).

Northern blot analysis, performed as described above, revealed that Tango-74 is expressed as an approximately 4.0 kb transcript in heart, brain, lung, liver, kidney, pancreas, spleen, prostate, testes, ovary, small intestine, colon and peripheral blood lymphocytes. Higher expression was seen in lung, liver, skeletal muscle, spleen, testes, colon and peripheral blood lymphocytes.

The amino acid sequence of Tango-74 is homologous to the amino acid sequence of the TRAIL receptor (Pan et al., *Science* 276:111, 1997) (FIG. 5).

Tango-76 cDNA (SEQ ID NO:7) was isolated from an adult rat frontal cortex library. The amino acid sequence of Tango-76 is homologous to the amino acid sequence of ADAMTS-1 (FIG. 14).

Northern blot analysis of human mRNA probed with a Tango-76 probe revealed a 4.2 kb band in lung. Analysis of rat mRNA revealed a weak 3.8 kb transcript in heart, brain, spleen, liver, skeletal muscle, and kidney and a weak 1.8 kb transcript in spleen and liver.

Effective Dose

Toxicity and therapeutic efficacy of the polypeptides of the invention and the compounds that modulate their expression or activity can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the

- 50 -

population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . Polypeptides or other compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

10 The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays.

20 A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used

25 to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Formulations and Use

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either

- 51 -

through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

- 52 -

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with

- 53 -

suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

5 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be
10 accompanied by instructions for administration.

 The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients which can be used include buffers (for example, citrate buffer, phosphate
15 buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or
20 modulatory compounds of the invention can be administered by any standard route of administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal,
25 intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be
30 made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that the preferred route of administration will be intravenous.

- 54 -

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule comprising a
5 nucleotide sequence which is at least 55% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof;
- 10 b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, or a
15 complement thereof;
- c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid
20 deposited with ATCC as Accession Number _____;
- d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, wherein the fragment comprises at least 15
25 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____; and
- e) a nucleic acid molecule which encodes a
30 naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the nucleic

- 55 -

acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions.

- 5 2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of:
 - a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the
10 plasmid deposited with ATCC as Accession Number _____, or a complement thereof; and
 - b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino
15 acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____.
3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.
4. The nucleic acid molecule of claim 1 further
20 comprising nucleic acid sequences encoding a heterologous polypeptide.
5. A host cell which contains the nucleic acid molecule of claim 1.
6. The host cell of claim 5 which is a mammalian
25 host cell.
7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

- 56 -

8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

- 57 -

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____;

b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising:

a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and

- 58 -

b) determining whether the compound binds to the polypeptide in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

5 15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising
10 the steps of:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or
15 primer binds to a nucleic acid molecule in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively
20 hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of:

25 a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and

b) determining whether the polypeptide binds to the test compound.

- 59 -

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detecting of
5 test compound/polypeptide binding;
- b) detection of binding using a competition binding assay;
- c) detection of binding using an assay for
Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-
10 mediated signal transduction.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a
15 sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising:

- 20 a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

GTGACCCACGGCTCCGAGCGGCTCCGAGCCAGGGGCTATTGCAAAACCCAGGGTGCGCTACCGGACGGAGAGGGGAGAG 79
 CCTGAGCAGAGTGAACATCGCAGCCAGGGGAGGCGGAAGAGGGGGCCAGGCCAATCTCCGGCTTGCGCTCA 158
 CCCCCGAGGGGGCCCCAGAGGGCTTCTGTGCCACAGAGCCACTCTGCGCTGGCGCTGCGCTCTCAGTGTCTCCAACTTT 237
 GCGCTGGAAGAAAACTTCCCGCGCGCGGAGAACTGCAGCGGCTCTCTTAGTAGACTCCGGGAGCTTCCGCTGTAGC 316
 CGGCTCTCGCGGCGCTTCCAAACGAATAATAGAAATGTTAATTTTAAACAATCCAGAGCAGGCCAACGAGGCTTTGCTCT 395
 CCGGACCCGAACTAAAGCTCCCTCGCTCCGTCGGCTGCTACGAACGGTGTCTCCTGGGGCTCCA M Q R 3
 ATG CAG CGA 468
 A V P E G F G R R K L G S D M G N A E R 23
 GCT GTG CCC GAG GGG TTC GGA AGG CGC AAG CTG GGC AGC GAC ATG GGG AAC GCG GAG CGG 528
 A P G S R S F G P V P T L L L L A A A L 43
 GCT CCG GGG TCT CCG AGC TTT GGG CCC GTA CCC ACG CTG CTG CTG CTC GCC GCG GCG CTA 588
 L A V S D A L G R P S E E D E E L V V P 63
 CTG GCC GTG TCG GAC GCA CTC GCG CGC CCC TCC GAG GAG GAC GAG GAG CTA GTG GTG CCG 648
 E L E R A P G H G T T R L R L H A F D Q 83
 CAG CTG GAG CGC GCC CCG GGA CAC GGG ACC ACG CGC CTC CGC CTG CAC GCC TTT GAC CAG 708
 Q L D L E L R P D S S F L A P G F T L Q 103
 CAG CTG GAT CTG GAG CTC CCG CCC GAC AGC AGC TTT TTG GCG CCC GGC TTC ACG CTC CAG 768
 N V G R K S G S E T P L P E T D L A H C 123
 AAC GTG GGG CGC AAA TCC GGG TCC GAG ACG CGC CTT CCG GAA ACC GAC CTG GCG CAC TCC 828
 F Y S G T V N G D P S S A A A L S L C E 143
 TTC TAC TCC GGC ACC GTG AAT GGC GAT CCC AGC TCG GCT GCC GCC CTC AGC CTC TGC GAG 888
 G V R G A F Y L L G E A Y F I Q P L P A 163
 GGC GTG CGC GGC GCC TTC TAC CTG CTG GGC GAG GCG TAT TTC ATC CAG CCG CTG CCC GCC 948
 A S E R L A T A A P G E K P P A P L Q F 183
 GCC AGC GAG CGC CTC GCC ACC GCC GCC CCA GCG GAG AAG CCG CCG GCA CCA CTA CAG TTC 1008
 H L L R R N R Q G D V G G T C G V V D D 203
 CAC CTC CTG CCG CGG AAT CCG CAG GGC GAC GTA GGC GGC ACG TGC GCG GTC GTG GAC GAC 1068
 E P R P T G R A E T E D E D E G T E G E 223
 GAG CCC CCG CCG ACT GCG AAA GCG GAG ACC GAA GAC GAG GAC GAA GCG ACT GAG CGC GAG 1128
 D E G P Q W S P Q D P A L Q G V G Q P T 243
 GAC GAA GGG CCT CAG TGG TCG CCG CAG GAC CCG GCA CTG CAA GGC GTA GGA CAG CCC ACA 1188
 G T G S I R K K R F V S S H R Y V E T M 263
 GGA ACT GGA AGC ATA AGA AAG AAG CCA TTT GTG TCC AGT CAC CGC TAT GTG GAA ACC ATG 1248
 L V A D Q S M A E F H G S G L K H Y L L 283
 CTT GTG GCA GAC CAG TCG ATG GCA GAA TTC CAC GGC AGT GGT CTA AAG CAT TAC CTT CTC 1308

FIGURE 1 (1 of 4)

T L P S V A A R L Y K H P S I R N S V S 303
ACG TTG TTT TCG GTG GCA GCC AGA TTG TAC AAA CAC CCC AGC ATT COT AAT TCA GTT AGC 1368

L V V V K I L V I H D E Q K G P E V T S 323
CTG GTG GTG GTG AAG ATC TTG GTC ATC CAC GAT GAA CAG AAG GGG CCG GAA GTG ACC TCC 1428

N A A L T L R N F C N W Q K Q H N P P S 343
AAT GCT GCC CTC ACT CTG CCG AAC TTT TGC AAC TGG CAG AAG CAG CAC AAC CCA CCC AGT 1488

D R D A E H Y D T A I L F T R Q D L C G 363
GAC CCG GAT GCA GAG CAC TAT GAC ACA GCA ATT CTT TTC ACC AGA CAG GAC TTG TGT GGG 1548

S Q T C D T L G M A D V G T V C D P S R 383
TCC CAG ACA TGT GAT ACT CTT GGG ATG GCT GAT GTT GGA ACT GTG TGT GAT CCG AGC AGA 1608

S C S V I E D D G L Q A A P T T A H E L 403
AGC TGC TCC GTC ATA GAA GAT GAT GGT TTA CAA GCT GCC TTC ACC ACA GCC CAT GAA TTA 1668

G H V F N M P H D D A K Q C A S L N G V 423
GGC CAC GTG TTT AAC ATG CCA CAT GAT GAT GCA AAG CAG TGT GCC AGC CTT AAT GGT GTG 1728

N Q D S H M M A S M L S N L D H S Q P W 443
AAC CAG GAT TCC CAC ATG ATG GCG TCA ATG CTT TCC AAC CTG GAC CAC ACC CAG CCT TGG 1788

S P C S A Y M I T S F L D N G H G E C L 463
TCT CCT TGC AGT GCC TAC ATG ATT ACA TCA TTT CTG GAT AAT GGT CAT GGG GAA TGT TTG 1848

M D K P Q N P I Q L P G D L P G T S Y D 483
ATG GAC AAG CCT CAG AAT CCC ATA CAG CTC CCA GGC GAT CTC CCT GGC ACC TCG TAC GAT 1908

A N R Q C Q P T F G E D S K H C P D A A 503
GCC AAC CCG CAG TCC CAG TTT ACA TTT GGG GAG GAC TCC AAA CAC TGC CCC GAT GCA GCC 1968

S T C S T L W C T G T S G G V L V C Q T 523
AGC ACA TGT AGC ACC TTG TGG TGT ACC GGC ACC TCT GGT GGG GTG CTG GTG TGT CAA ACC 2028

K H F P W A D G T S C G E G K W C I N G 543
AAA CAC TTC CCG TGG GCG GAT GGC ACC AGC TGT GGA GAA GGG AAA TGG TGT ATC AAC GGC 2088

K C V N K T D R K H F D T P F H G S W G 563
AAG TGT GTG AAC AAA ACC GAC AGA AAG CAT TTT GAT ACG CCT TTT CAT GGA AGC TGG GGA 2148

M W G P W G D C S R T C G G G V Q Y T M 583
ATG TGG GGG CCT TGG GGA GAC TGT TCG AGA ACC TGC GGT GGA GGA GTC CAG TAC ACG ATG 2208

R E C D N P V P K N G G K Y C E G K R V 603
AGG GAA TGT GAC AAC CCA GTC CCA AAG AAT GGA GGG AAG TAC TGT GAA GGC AAA CCA GTG 2268

R Y R S C N L E D C P D N N G K T F R E 623
CGC TAC AGA TCC TGT AAC CTT GAG GAC TGT CCA GAC AAT AAT GGA AAA ACC TTT AGA GAG 2328

E Q C E A H N E F S K A S F G S G P A V 643
CAA CAA TGT GAA GCA CAC AAC GAG TTT TCA AAA GCT TCC TTT GGG AGT GGG CCT GCG GTG 2388

E W I P K Y A C V S P K D R C K L I C Q 663
GAA TGG ATT CCC AAG TAC GCT GGC GTC TCA CCA AAG GAC AGG TGC AAG CTC ATC TGC CAA 2448

A K G I G Y F F V L Q P K V V D G T P C 683

GCC AAA GGC ATT GGC TAC TTC TTC GTT TTG CAG CCC AAG GTT GGA GAT GGT ACT CCA TGT 2508
S P D S T S V C V Q G Q C V K A G C D R 703
AGC CCA GAT TCC ACC TCT GTC TGT GTG CAA GGA CAG TGT GTA AAA GCT GGT TGT GAT CGC 2568
I I D S K K K F D K C G V C G G N G S T 723
ATC ATA GAC TCC AAA AAG AAG TTT GAT AAA TGT GGT GTT TGC GGG GGA AAT GGA TCT ACT 2628
C K R I S G S V T S A K P G Y H D I I T 743
TGT AAA AAA ATA TCA GGA TCA GTT ACT AGT GCA AAA CCT GGA TAT CAT GAT ATC ATC ACA 2688
I P T G A T N I E V K Q R N Q R G S R N 763
ATT CCA ACT GGA GCC ACC AAC ATC GAA GTG AAA CAG CGG AAC CAG AGG GGA TCC AGG AAC 2748
N G S F L A I K A A D G T Y I L N G D Y 783
AAT GGC AGC TTT CTT GCC ATC AAA GCT CCT GAT GGC ACA TAT ATT CTT AAT GGT GAC TAC 2808
T L S T L E Q D I M Y K G V V L R Y S G 803
ACT TTG TCC ACC TTA GAG CAA GAC ATT ATG TAC AAA GGT GTT GTC TTG AGG TAC AGC GGC 2868
S S A A L E R I R S F S P L K E P L T I 823
TCC TCT CGG GCA TTG GAA AGA ATT CGC AGC TTT AGC CCT CTC AAA GAG CCC TTG ACC ATC 2928
Q V L T V G N A L R P K I K Y T Y F V K 843
CAG GTT CTT ACT GTG GGC AAT GCC CTT CGA CCT AAA ATT AAA TAC ACC TAC TTC GTA AAG 2988
K K K E S F N A I P T F S A W V I E E W 863
AAG AAG AAG GAA TCT TTC AAT GCT ATC CCC ACT TTT TCA GCA TGG GTC ATT GAA GAG TCG 3048
G E C S K S C E L G W Q R R L V E C R D 883
GGC GAA TGT TCT AAG TCA TGT CAA TTG GGT TGG CAG AGA AGA CTG GTA GAA TGC CGA GAC 3108
I N G Q P A S E C A K E V K P A S T R P 903
ATT AAT GGA CAG CCT GCT TCC GAG TGT GCA AAG GAA GTG AAG CCA GCC AGC ACC AGA CCT 3168
C A D H P C P Q W Q L G E W S S C S K T 923
TGT GCA GAC CAT CCC TGC CCC CAG TGG CAG CTG GGG GAG TGG TCA TCA TGT TCT AAG ACC 3228
C G K G Y K K R S L K C L S H D G G V L 943
TGT GGC AAG GGT TAC AAA AAA AGA AGC TTG AAG TGT CTG TCC CAT GAT GGA GGG GTG TTA 3288
S H E S C D P L K K P K H F I D F C T M 963
TCT CAT GAG AGC TGT GAT CCT TTA AAG AAA CCT AAA CAT TTC ATA GAC TTT TGC ACA ATG 3348
A E C S * 968
GCA GAA TGC AGT TAA 3363
GTGGTTAAGTGGTCTTACCTTTGAGGGCAAGGCAAGTGAGGAAGGGCTGGTGCAGGGAAAGCAAGAAGGCTGGAGGG 3442
ATCCAGCGTATCTTGCCAGTAACCAAGTGAGCTGTATCAGTAAGCGTGGGATTATGGGGGTAGATAGAAAAGGAGTTGAAT 3521
CATCAGAGTAAGTGGCAGTTGCAAAATTTGATAGCATAGTTTASTGAGGATTTATTAACCTCTGAGCAGTGATATAGCATA 3600
ATAAAGCCCCGGGCATTATTATTATTATTTCTTTTGTACATCTATTACAAGTTTAGAAAAACAAAGCAATTGTCAAA 3679
AAAAGTTAGAACTATTACAACCCCTGTTTCCTGGTACTTATCAATACTTAGTATCATGGGGGTGGGAATGAAAAGT 3758
AGGAGAAAAGTGAGATTTTACTAAGACCTGTTTACTTTTACCTCACTAACANTGGGGGAGAAAGGAGTACAAATAGGA 3837

TCTTTGACCAGCACTGTTTATGGCTGCTGTGGTTTCAGAGAATGTTTATACATTATTTCTACCGAGAATTAAACTTCA 3916
GATTGTTCAACATGAGAGAAAGGCTCAGCAACGTGAATAACGCCAAATGGCTTCCTCTTCTCTTTTGGACCATCTCA 3995
GTCTTTATTTGTGTAATTCATTTTGAGGAAAAACAACCTCCATGTATTTATTCAGGTGCATTAAAGTCTACAATGGAAA 4074
AAAAGCAGTGAAGCAATTACATGCTGGTAAAAGCTAGAGGAGACACATGAGCTTAGTACCTCCAACCTTCTTTCTTCC 4153
TACCATGTAAACCTGCTTTCCGAATATGGATGTAAAGAAGTAACCTGTGTCTCATGAAAATCAGTACAATCACAAGG 4232
AGGATGAAACCCCGAACAAAATGAGGTGTGTAGAACAGGGTCCCAAGGTTTGGGGACATTGAGATCACTTGTCTTG 4311
TGGTGGGAGGCTGCTGAGGGGTAGCAGGTCCATCTCCAGCAGCTGGTCCAACAGTGGTATCCTGGTGAATGTCTGTT 4390
AGCTCTCTGTGAGAAATATGATTTTTCCATATGTATATAGTAAATATGTTACTATAAATTACATGTACTTTATAAGT 4469
ATTGGTTTGGGTGTTCTTCCAAGAGGACTATAGTTAGTAATRAATGCCATATAAATACATATTTATTTTATACATT 4548
ATTTCDAATGAAAAAACTTTTAAATTATATGCTTTTGTGGAGTGCATATAAAATAGAGTATTTATACAATATATGT 4627
TACTAGAAATAAAAGAACCTTTTGGAAAAAATAAAAAAAAAAAAAA 4676

GTGACCCAGCGGTCCGGGGAGCAACCCAGCTTCTAGTATCCAGACTCCAGGGGGGGGGGGGGGGGACCCCAACCC 79
CGACCCAGAGCTTCTCCAGGGGGGGGGAGCGGAGCAAGGGCTCCCGGGCTTAACCTTCTCCGGGGGGGGGGAGCCACTTC 158
GGGAGTCCGGGTTCGCCACCTGCAAACTCTCCGCTTCTGCACCTGCCACCCCTGAGCCAGGGGGGGGGGGAGCGGAG 237
M A N A G L Q L L G F I L A F L G W I 19
TC ATG GCC AAC GCG GGG CTG CAG CTG TTG GGC TTC ATT CTC GCC TTC CTG GGA TGG ATC 296
G A I V S T A L P Q W R I Y S Y A G D N 39
GCC GCC ATC GTC AGC ACT GCC CTG CCC CAG TGG AGG ATT TAC TCC TAT GCC GGC GAC AAC 356
I V T A Q A M Y E G L W M S C V S Q S T 59
ATC GTG ACC GCC CAG GCC ATG TAC GAG GGG CTG TGG ATG TCC TGC GTG TCG CAG AGC ACC 416
G Q I Q C K V F D S L L N L S S T L Q A 79
GGG CAG ATC CAG TCC AAA GTC TTT GAC TCC TTG CTG AAT CTG AGC AGC ACA TTG CAA GCA 476
T R A L M V V G I L L G V I A I F V A T 99
ACC COT GCC TTG ATG GTG GTT GGC ATC CTC CTG GGA GTG ATA GCA ATC TTT GTG GCC ACC 536
Y G M K C M K C L E D D E V Q K M R M A 119
TTC GCC ATG AAG TGT ATG AAG TGC TTG GAA GAC GAT GAG GTG CAG AAG ATG AGG ATG GCT 596
V I G G A I F L L A G L A I L V A T A W 139
GTC ATT GGG GGT GCG ATA TTT CTT CTT GCA GGT CTG GCT ATT TTA GTT GCC ACA GCA TGG 656
Y G N R I V Q E F Y D P M T P V N A R Y 159
TAT GCC AAT AGA ATC GTT CAA GAA TTC TAT GAC CCT ATG ACC CCA GTC AAT GCC AGG TAC 716
E F G Q A L F T G W A A A S L C L L G G 179
GAA TTT GGT CAG GCT CTC TTC ACT GGC TGG GCT GCT GCT TCT CTC TGC CTT CTG GGA GGT 776
A L L C C S C P R K T T S Y P T P R P Y 199
GCC CTA CTT TGC TGT TCC TGT CCC CGA AAA ACA ACC TCT TAC CCA ACA CCA AGG CCC TAT 836
P K P A P S S G K D Y V * 212
CCA AAA CCT GCA CCT TCC AGC GGG AAA GAC TAC GTG TGA 875
CACAGAGCCAAAAGGAGAAAATCATGTTGAACAACCGAAAATGGACATTGAGATACTATCATTAACATTAGGACCTT 954
AGAAATTTGGGTATTGTAACTCTGAAGTATGGTATTACAAAACAACAACAACAAAACCCATGTGTTAAATACTC 1033
AGTGCTAAACATGGCTTAATCTTATTTTATCTTCTTCTCAATATAGGAGGGAAGATTTTCCATTGTATTACTGCT 1112
TCCCATTGAGTAATCATCTCACTGGGGGAAGGGGTGCTCTTAAATATATATAGATATGTATATATACATGTTTTTC 1191
TATTAATAATAGACAGTAAATACTATTCTCATTATGTTGATACTAGCATACTTAAATATCTCTAAAATAGGTAAATG 1270
TATTTAATTCATATGTATGAAGATGTTTATGTTATATTTTCTTTTCTGCTATATATACATATGTAAACAGTCAAATA 1349
TCATTTACTCTTCTTCACTAGCTTTGGGTGCCCTTGGCCACAAGACCTAGCCTAATTTACCAAGGATGAATTCCTTCAAT 1428
TCTTCATGGGTGCCCTTTCAATACTTATTTTATTTTACCATAATCTTATAGCACTTGCATCGTTATTAAGCCCTT 1507
ATTGTTTTGTGTTTCACTGGTCTCTATCTCTGAATCTAACACATTTTCATAGCCTACATTTTATGTTTCTAAAGCCCAAG 1586
AAGAATTTATTACAAATCAGAACTTTGGAGGCAAACTTTCTGCAATGACCAAGTGATAAATTCCTGTTGACCTTCCCA 1665

Fig. 2 (1 of 2)

CACAATCCCTGACTCTGACCCATAGCACTCTTGTTCCTTTGAAAATATTGTCCAAATTGAGTAGCTGCATGCTGTTTC 1744
CCCCAGGTGTGTAAACACAACCTTTATGATTGAATTTTAAGCTACTTATTCTAGTTTTATATCCCCCTAAACTACCT 1323
TTTGTTCCTCCATCTCTAATTGTATTGTTTTCCCAAGTGTAAATATCARGCGTTTATATCTTCCCTAATAAGGTGTGG 1902
TCTGTTTCTCTGAACAAAGTGTAGACTTTCTGGAGTGATAATCTGCTGACAAATATTCTCTCTAGCTGTAAAGCAAG 1981
TCACTTAATCTTTCTACCTCTTTTTCTATCTGCCAAATTGAGATAATGATACTTAACCACTTAGAAGAGGTAGTGTGA 2060
ATATTAATTAGTTTATATTACTCTCATTCTTTGAACATGAACATATGCCATGTAGTGTCTTTATTGCTCAGCTGGCTG 2139
AGACACTGAAGAAGTCACTGAACAAAACCTACACACGTACCTTCATGTGATTCACTGCCTTCTCTCTACCAGTCTA 2218
TTTCCACTGAACAAAACCTACACACATACCTTCATGTGGTTCAGTGCCTTCTCTCTACCAGTCTATTTCCTACTGAA 2297
CAAAACCTAGGCACATACCTTCATGTGGCTCAGTGCCTTCTCTCTCTACCAGTCTATTTCCTCATTTCTTCAGCTGTGTC 2376
TGACATGTTTGTGCTCTGTTCATTTTAACTGCTCTTACTTTTCCAGTCTGTACAGAATGCTATTTCCTCTGAGCA 2455
AGATGATGTAATGGAAAGGTGTGGCATTGCTGTCTGGAGACCTGCAATTTGAGTCTTGGTGGCTATCAATCACCGTCTG 2534
TGTTTGAGCAAGGCATTTGGCTGCTGTAAAGCTTATTGCTTCATCTGTAAAGCGGTGGTTTGTAAATCTCTGATCTTCCAC 2613
CTCACAGTGATGTTGTGGGGATCCAGTGAGATAGAAATACATGTAAAGTGTGGTTTGTAAATTTAAAAGTGCTATACTAA 2592
GGGAAGAATTGAGGAATTAAGTGCATACGTTTGGTGTGCTTTTCAAATGTTTGAAAACAAAAAAATGTTAAGAAA 2771
TGGGTTCTTGGCTTAACCACTCTCTCAAGTGATGAGACAGTGAAGTAAAATTGAGTGCACTAAACAAATAAGATTCTG 2850
AGGAAGTCTTATCTTCTCGAGTGAGTATGCCCCGATGCTTTCTGTGGCTAAACAGATGTAATGGGAAGAAATAAAGCC 2929
TAGTGTGTGTAATCCACAGCAAGGGAGATTTTGAATCATAATAACTCATAAGGTGCTATCTGTTCACTGATGCCC 3008
TCAGAGCTCTTCTGTAGCTGGCAGCTGACCGTCTAGGATAGTATGTTTGGAAATGGTACTTCATAATAAACTACAC 3087
AAGGAAAGTCAGCCACTGTGTCTTATGAGGAATTGGACCTAATAAATTTTATGTGCTTCCAAACCTGAGAAATATATG 3166
CTTTTGGAGTTAAATTTAAATGGCTTTTGCCACATACATAGATCTTCATGATGTGTGAGTGTAAATCCATGTGGATA 3245
TCAGTTACCAACATTACAAAAAATTTTATGGCCCAAAATGACCAACGAAATGTTACAATAGAATTTATCCAATTTT 3324
GATCTTTTATATTCTTCTACCACACCTGGAACAGACCAATAGACATTTTGGGGTTTTATAATAGGAATTTGTATAAA 3403
GCATTACTCTTTTCAATAAATGTGTTTTTAATTTAAAAAAGGAAAAAAAAAAAAAAAAAAAAAAAAAAGGGGGGGCG 3482

3483

C

Fig. 2 (2 of 2)

TTGGAGCCGACGCTTCCGGCTGCGAGAACCTTTCCAGCCGCAAACTACGGGGACGATTTCGTGATTGATTTTGGCGCT 79
 M G L W G Q S V P T A S S 13
 TTGGATCCGACGCTTCCGGCTGCGAGAACCTTTCCAGCCGCAAACTACGGGGACGATTTCGTGATTGATTTTGGCGCT 142
 A R A G R Y P G A R T A S G T R P W L L 33
 GCT CGA GCA GGG CGC TAT CCA GGA GCC AGG ACA GCG TCG GGA ACC AGA CCA TGG CTC CTC 102
 D E K I L K F V V F I V A V L L P V R V 53
 GAC TCG AAG ATC CTT AAG TTC GTC GTC TTC ATC GTC GCG GTT CTG CTG CCG GTC CGG GTT 162
 D E A T I P R Q D E V P Q Q T V A P Q Q 73
 GAC TCT GCC ACC ATC CCC CGG CAG GAC GAA GTT CCC CAG CAG ACA GTG GCC CCA CAG CAA 122
 Q R R S L K E E E C P A G S H R S E Y T 93
 CAG AGG CGC AGC CTC AAG GAG GAG GAG TGT CCA GCA GGA TCT CAT AGA TCA GAA TAT ACT 182
 S A C N P C T E G V D Y T I A S N N L P 113
 GGA GCG TGT AAC CCG TGC ACA GAG GGT GTG GAT TAC ACC ATT GCT TCC AAC AAT TTG CTT 142
 S D L L C T V C K S G Q T N K S S C T T 133
 TCT TCC CTG CTA TGT ACA GTT TGT AAA TCA GGT CAA ACA AAT AAA AGT TCC TGT ACC ACG 162
 T R D T V C Q C E K G S F Q D K N S P E 153
 ACC AGA GAC ACC GTG TGT CAG TGT GAA AAA GGA AGC TTC CAG GAT AAA AAC TCC CCT GAG 162
 M C R T C R T G C P R G M V K V S N C T 173
 ATG TGC CGG ACG TGT AGA ACA GGG TGT CCC AGA GGG ATG GTC AAG GTC AGT AAT TGT ACG 182
 P R S D I K C K N E S A A S S T G K T P 193
 CCC CGG AGT GAC ATC AAG TGC AAA AAT GAA TCA GCT GCC AGT TCC ACT GGG AAA ACC CCA 182
 A A E E T V T T I L G M L A S P Y H Y L 213
 GCA GCG GAG GAG ACA GTG ACC ACC ATC CTG GGG ATG CTT GCC TCT CCC TAT CAC TAC CTT 142
 I I I V V L V I I L A V V V V G F S C R 233
 ATC ACC ACA GTG GTT TTA GTC ATC ATT TTA GCT GTG GTT GTG GTT GGC TTT TCA TGT CCG 182
 K E F I S Y L K G I C S G G G G G P E R 253
 AAG AAA TTC ATT TCT TAC CTC AAA GGC ATC TCC TCA GGT GGT GGA GGA GGT CCC GAA CTT 162
 V H R V L F R R R S C P S R V P G A E D 273
 GTG CAC AGA GTC CTT TTC CGG CGG CGT TCA TGT CCT TCA CGA GTT CCT GGG GCG GAG GAC 122
 N A R N E T L S N R Y L Q P T Q V S E Q 293
 AAT GCC CGC AAC GAG ACC CTG AGT AAC AGA TAC TTG CAG CCC ACC CAG GTC TCT GAG CAG 182
 E I Q G Q E L A E L T G V T V E S P E E 313
 GAA ATC CAA GGT CAG GAG CTG GCA GAG CTA ACA GGT GTG ACT GTA GAG TCG CCA GAG GAG 1042
 P Q R L L E Q A E A E G C Q R R R L L V 333
 CCA CAG CGT CTG CTG GAA CAG GCA GAA GCT GAA GGG TGT CAG AGG AGG AGG CTG CTG GTT 1102
 P V N D A D S A D I S T L L D A S A T L 353
 CCA GTG AAT GAC GCT GAC TCC GCT GAC ATC AGC ACC TTG CTG GAT GCC TCG GCA ACA CTG 1162
 E E G H A K E T I Q D Q L V G S E K L F 373
 GAA GAA GCA CAT GCA AAG GAA ACA ATT CAG GAC CAA CTG GTG GGC TCC GAA AAG CTC TTT 1222

FIG. 3 (sheet 1 of 2)

Y E E D E A G S A S C L * 387
 TAT GAA GAA GAT GAA GCA GGC TGT GCT ACG TCC TGC CTG TGA 1264

AAGAATCTCTTCAGGAAACCAGAGCTTCCCTCATTTACCTTTTCTCTACAAAGGGAAGCAGCCTGGAGAAACAGTCC 1343
 AGTACTTACCCCTGCCCAACAACTCTACTATCCAAATATGGGGCAGCTTACCAATGGTCTTAGAAGCTTTGTAAAGC 1422
 ACTTGGAGTAATTTTATGAAATACTGCGTGTGATAAGCAAACGGGAGAAATTTATATCAGATTCTTGGCTGCATAGTT 1501
 ATACGATTGTATTAAGGGTCTTTTAGGCCACATGCCGTGGCTCATGCCCTGTAATCCCAAGCCTTTGATAGGCTGAG 1580
 TCAAGTGGATTCTCTGAGCTCGGGAGTTTGAGACCAGCCTCATCAACACAGTGAAACTCCATCTCAATTTAAAAAGAAA 1659
 AAAAGTGGTTTATAGGATGTCAATCTTTGCAGTTCTTCATCATGAGACAAGTCTTTTCTGCTCTTTATATTGCAAGC 1738
 TCCATCTCTACTGGTGTGTGCTTTAATGACATCTAACTACAGATGCCGCACAGCCACAATGCTTTGCCCTATAATTTT 1817
 TTAAGTTTGAACGGGATTAATCTGTATTACCTGTATTTTCAGTTTCGGATATTTTGAAGTTAATGATGAGATTATCA 1896
 AGACGTAGCCCTATGCTAAGTCATGAGCATATGGACTTACGAGGGTTCGACTTAGAGTTTGAAGTCTTAAGATACGATT 1975
 ATTGGGGCTTACCCCTCTTAATTAGAGAAACATTTATATGCTTACTACTGTAGGCTGTACATCTCTTTTCCGATTT 2054
 TTTATATAGTGTAAACATGGAAAACTTTAGGAAATGCATTTATAGGCTGTTCATGGGTTCCTGGATACAAAT 2133
 TCCAGTCAAAAATGACTAAAAATATACTAGTGACGGGAGGAGAAATCCCTCCCTCTGTTGGGAGGCACTTACTGCATTC 2212
 CAGTTCTCTCTCTGGCCCTGAGACTGGACCAGGGTTTGATGGCTGGCAGCTTCTCAAGGGGAGCTTGTCTTACTTG 2291
 TTAATTTAGAGGTATATAGCCATATTTATTTATAAATAAATATTTATTTATTTATTAAGTAGATGTTTACATATG 2370
 CCCAGGATTTGAAGAGCCCTGGTATCTTTGGGAAGCCATGTGTCTGGTTTGTCTGCTGGGACAGTCATGGGACTGCAT 2449
 CTTCCGACTTGTCCACAGCAGATGAGGACAGTGAGAATTAGTTAGATCCGAGACTGCGAAGAGCTTCTCTTTCAAGCG 2528
 CCATTACAGTTGAACGTTAGTGAATCTTGAGCCTCATTTGGGCTCAGGGCAGAGCAGGTGTTTATCTGCCCGGCATCT 2607
 GCCATGGCATCAAGAGGGAAGAGTGGACGGTGTCTGGGAATGGTGTGAAATGGTTGCCGACTCAGGCATGGATGGGCCC 2686
 TCTCCCTCTCTGGTGTCTGTAAGTCTGAGTCCCTGGGATGCCCTTTAGGGCAGAGATTCTGAGCTGGCTTTTAGGTA 2765
 TCAATTCCTCTTTGAGGAGCTTGGCCCTCTGTAAAGCATCTGACTCATCTCGAGATATCAATCTTTAAACACTGTGA 2844
 CACAGGATCTAAATGGCTGACACATTTGTCTCTGTGTACGTTCCATTATTTATTTAAAAACCTCAGTAATCGTTT 2923
 TAGCTCTTTTCCAGCAACTCTTCTCCACAGTAGCCCTGCTGGTATAGGATAAAATTACGGATATAGTCATTCTAGGGGT 3002
 TTCAGTCTTTTCCATCTCAAGGCATTTGTGTGTTTGTTCGGGACTGGTTTGGCTGGGACAAAGTTAGAAGTGCCTGAA 3081
 GTTCGCACATTCAGATTGTTGTGTCCATGGAGTTTATAGGAGGGATGGCCTTTCCGGTCTTCCGACTTCCATCTCTCC 3160
 CACTTCCATCTGGCGTCCACACCTTGTCCCTGCACTTCTGGATGACACAGGGTGTCTGCTGCTCTAGTCTTTGCCT 3239
 TTGCTGGGCTCTGTGTCAGGAGACTTGGTCTCAAAGCTCAGAGAGAGCCAGTCCGGTCCAGCTCTCTTGTCCCTTCC 3318
 TCAGAGGCTTCTTTGAAGATGCATCTAGACTACCAGCCTTATCAGTGTTTAAGCTTATTCCTTTAACAATAAGCTTCTT 3397
 GACAACATGAAATGTTGGGGTTTTTGGCGTTGGTTGAATGTTTAGGTTTGTCTTTATACCCGGGCCAATAGCACA 3476
 TAAACCTGGTTATATATGAAATACTCATATGTTTATGACCAAATAAATATGAAACCTCATATTAACAAAAA 3555
 AAAAGGGGGGGGG 3569

GOCITTTAGGGCAGAGATTCTGAGCTGCGTTTTAGGGTACAGATTOCT
GTTTGAGGAGCTTGGOOCTCTGTAAAGCATCTGACTCATCTCAGAGATAT
CAATTCCTAAACACTGTGACAACAGGATCTAAAATGGCTGACACATTTGT
OCTTGTGTCAOGTTCATTATTTATTTAAAAAOCCTCAGTAATCGTTTTA
GCTTCITTOCAGCAAACCTCTCTOCACAGTAGOOOCAGTCTGTGGTAGGATA
AATTAOGGATATAGTCATTCTAGGGGTTTCAGTCTTTTTCATCTCAAGGC
ATTGTGTGTTTTGTDOGGGACTGGTTTTGGCTGGGACAAAGTTAGAACTG
OCTGAAGTTOGCACATTCAGATTGTTGTGTTCATGGAGTTTTAGGAGGGG
ATGGOCTTTTOGGTCTTOGCACITOCATCTCTOOEACTTOCATCTGGOG
TOOCACAOCTTGTDOOCTGCACTTCTGGATGACACAGGGTGCTGCTGOCT
OCTAGTCTTTGOCITTTGCTGGGOCITCTGTGCAGGAGACTTGGTCTCAA
GCTCAGAGAGAGOCAGTDOGGTOOCAGCTOCTTTGTDOCTTOCTCAGAGG
OCTTOCTTGAAGATGCATCTAGACTAOCAGOCCTATCAGTGTTTAAGCTT
ATTOCTTTAACATAAGCTTOCTGACAACATGAAATTGTTGGGGTTTTTTG
GOGTTGTTGATTTGTTTAGGTTTTGCTTTATAOCCGGGOCAAATAGCAC
ATAACAOCTGGTTATATATGAAATACTCATATGTTTATGAACAAAATAAA
TATGAAAOCTCATATTAACAAAAAAAAAAAAAAAAAGGGGOGGOG

Fig. 4

56.2% identity in 176 aa overlap

```

      30      40      50      60      70      80
TRAIL AAATPSKVGSSAGRIEPRGGGRGALPTSMGQHGPSA-RARAGRAPGPRPAREASPRLRV
      :: :: :::: :::: :::: ::::
T74      MGLMGQSVPTASSARAGRYPGARTASGTRPWLLD
      10      20      30

      90      100      110      120      130      140
TRAIL ECTIFKVV--VGVLQVVPSSAATIK---LHDQSIGTQQWEHSPLGELCPGSHRSERPG
      ::::: ::::: ::::: ::::: ::::: ::::: X: ::::: :::::
T74      SAILKRVVFIVAVLLFVRVDSATIPRQDEVPOQTVAPOQQRRLKEEECPAGSHRSEYTG
      40      50      60      70      80      90

      150      160      170      180      190      200
TRAIL ACNRCTEGVGYINASNLFACLPCTACKSDEEERSPCTTTTNTACQCKPGTFRNDSAEH
      :: ::::: ::::: ::::: ::::: ::::: :::::
T74      ACNPCTEGVDYTLASNLPSCLLATVCKSGQINRKSCTTTRDTVCQCKGSGFQDKNSPEM
      100      110      120      130      140      150

      210      220      230      240      250      260
TRAIL CRKCTGCPRGVVKVDCTPWSDEEC/HKESGVGHENIIVTLVTVLWVPLLLWAVLIVCCC
      :: ::::: ::::: ::::: X:
T74      CRTCTGCPRGVVKVSNCTPRSDIRCKNESAASTGCTPAAEETVTTILGLASPYHYLI
      160      170      180      190      200      210
```

Fig. 5

V R N K T L P W S P C S A V Y L T E L	19
GC GTC CCG AAC AAG ACG CTG CCC TGG TCT CCC TGC AGT GCT GTC TAC CTC ACG GAG CTC	59
L D D G H G D C L L D D G H S T L Y E L	39
CTG GAT GAT GGT CAC GGA GAC TGC CTC CTG GAT GAT GGC CAC AGC ACC CTC TAT GAG CTG	119
D Q Q C K Q I F G P D F R H C P N T S V	59
GAC CAG CAG TGC AAG CAG ATC TTT GGG CCT GAT TTC CGA CAC TGC CCC AAC ACC TCT GTG	179
E D I C V Q L W C R H R D S D E P I C H	79
GAG GAC ATC TGT GTC CAG CTC TGG TGC CGT CAT CCG GAT AGT GAT GAG CCC ATT TGC CAC	239
T K N G S L L W A - D G T P C G P G H L C	99
ACA AAG AAT GGC AGC TTG CTC TGG GCT GAT GGT ACG CCC TGT GGC CCT GGG CAC CTG TGC	299
L D G S C V L R E E V E N P K A V V D G	119
CTG GAT GGT AGC TGT GTG CTC CCG GAG GAA GTA GAG AAT CCC AAG GCT GTG GTA GAT GGA	359
D W G P W G P W G Q C S R T C G G G I Q	139
GAC TGG GGT CCC TGG GGA CCC TGG GGA CAA TGT TCT CCC ACC TGT GGT GGA GGG ATA CAG	419
F S N R E C D N P A P Q N G G R F C L G	159
TTT TGG AAC CTT GAG TGT GAT AAT CCA GCA CTT CAG AAT GGA GGA AGA TTT TGC CTG GGA	479
E R V K Y Q S C K T E E C P P N G K S F	179
GAG AGA GTC AAG TAC CAA TCT TGC AAG ACA GAG GAA TGT CCA CCA AAT GGA AAA AGC TTC	539
R E Q Q C E K Y N A Y N H T D L D G N F	199
AGG GAG CAG CAG TGT GAA AAA TAT AAT GCC TAC AAC CAC ACG GAC CTG GAT GGG AAT TTC	599
L Q W V P K Y S G V S P R D R C K L F C	219
CTT CAG TGG GTC CCC AAA TAC TCA GGA GTG TCC CCC CGA GAC CGA TGC AAA CTG TTT TGC	659
R A R G R S E F K V F E T K V I D G T L	239
AGA GCC CTT GGG AGG AGT CAG TTC AAA GTG TTT GAA ACT AAG GTG ATC GAT GGC ACT CTG	719
C G P D T L A I C V R G Q C V K A G C D	259
TGC GGA CCG GAT ACT CTG GCC ATC TGT GTG CCG GGA CAG TGC GTT AAG GCT GGC TGT GAC	779
H V V N S P K K L D K C G V C G G K G T	279
CAT GTG GTG AAC TCA CTT AAG AAG CTG GAC AAG TGT GGG GTG TGT GGG GGC AAA GGC ACT	839
A C R K V S G S F T P F S Y G Y N D I V	299
GCC TGT AGG AAG GTC TCA GGT TCT TTC ACC CCC TTC AGT TAT GGC TAC AAT GAC ATT GTC	899
T I P A G A T N I D V K Q R S H P G V Q	319
ACC ATC CCA GCT GGT GCC ACA AAT ATT GAT GTG AAA CAA CGG AGC CAC CCA GGG GTG CAG	959
N D G S Y L A L K T A N G Q Y L L N G N	339
AAT GAC GGC AGC TAC CTG GCA CTG AAG ACA GCC AAT GGG CAG TAC CTG CTC AAT GGT AAC	1019
L A I S A I E Q D I L M K G T I L K Y S	359
CTA GCC ATC TCT GCC ATA GAG CAA GAC ATC TTG ATG AAG GGG ACC ATC CTA AAG TAC AGT	1079
G S M A T L E R L Q S F Q A L P E P L T	379
GGT TCC ATG GCC ACC CTG GAG CCG CTG CAG AGC TTC CAA GCC CTC CCT GAG CCT CTT ACA	1139

Fig. 6 (1 of 2)

V Q L L T V S G E V F P P K V K Y T F F 399
GTA CAG CTC CTG ACT GTG TCT GGT GAG GTC TTC CCT CCA AAA GTC AAA TAT ACC TTC TTC 1299

V P N D T D F N V Q S S K E R A S T N I 419
GTC CCC AAT GAC ACG GAC TTC AAC GTG CAG AGT AGC AAA GAA AGA GCA AGC ACC AAC ATC 1259

I Q S L P Y A E W V L G D W S E C P S T 439
ATT CAG TCC TTG CCC TAT GCA GAG TGG GTG CTG GGG GAC TGG TCT GAA TGT CCA AGC ACA 1319

C G G G W Q R R T V E C R D P S G Q A S 459
TGT GGA GGT GGC TGG CAG CGG CGG ACT GTG GAA TGC AGG GAC CCC TCA GGT CAG GCC TCT 1379

D T C D E A L K P - E D A K P C G S Q P C 479
GAC ACC TGT GAT GAG GCT CTG AAA CCT GAG GAT GCC AAG CCC TGT GGA AGC CAG CCA TGT 1439

L L * 482
CTC CTC TGA 1448

TCCCTTGGTGGACATGTCTAAGGCTTATGGATTGGGGTACTGGCGTACAGACAAAGGTCTCCTCTGAGGTGACACTA 1527

CATATCAGATGGCATGGCCCTTCCCGCCCTTCTATTACTACAACCCCTTGGGTACCACTAATTCATAAGGAAGAGAG 1506

AAGAGGATGTAAGGGTAACAGACTGTAAAGTGTACTGTCTAGTGGACTGGACCTTGTTTATGACCAAGAAGATGGGATA 1585

GGTTAAAGGTAAAGTGTGCTTATTGATCCAAAGGTGAGATTTCAGAACCAGCCCTCTTTCCAAAGGACTAGAAAGGTT 1764

AAATGAGAAAGAAGAAATTTTCTCTCTTGGTTTCTCCAATAATCAATCTACCTCAGAGCGGGAGGAACCTTGGTGTAT 1843

AAGGCCAGGTGTTAGTGGTGAGTGCCAAAGGCACCTCTCCATAGATATCTTCGAGCCATCTTCAGAAATGGCCATGGCTGT 1922

TTTCAGTATTAAACTCTGTGTCTCAAAGGTGGTGGTGTCCATCAGGGTTATAGAAAGCCACTTGTCTCAGGCT 2001

GCCTCTCTCTGGGGCGGACCCCTTTCAGTATTTATGCAATATGTTTCTGAACTAAAGTGTGATCTTACACCAAAAAA 2080

AAAAAAAAAAAAAAAAAAAAAAAAAGGCGGCGC 2114

Fig. 6 (2 of 2)

>/thxe29c9F. 1448 bases, 3233 checksum.

GTGAGGCC*OGGTTGGGGGAAGCTTGCCAGCAGATCTGCAGCTGCCAA
AATGGGGCAGACTGTGACAGTGTGACTGGAAAGTGCAOCTGTGCCCCAGG
ATTCAAAGGAATTGACTGCTCTACCCCATGCOCTCTGGGAAOCTATGGGA
TAAACTGTTCCTCTGCTGTGGCTGTAAAAATGATGCAGTCTGCTCTOCT
GTGGAAGGGTCTTGTACTTGCAAGGCAGGCTGGCAOAGGGTGGACTGCTC
CATCAGATGTCCAGTGGCAGATGGGGCTTTGGCTGTAACTTAACATGOC
AGTGCTCAAGGGGGAGCTGCAACAOCCTGGAAGGGAGCTGCAOCTGT
GCAOCTGGATGGGGGGGAGAAATGCGAAGCTTCOCTGCCAGGATGGCAC
GTAAGGGCTGAAGTGTGCTGAGGGCTGGAGTGCAGOCAGCAGATGGCT
GOCACOCCTAOCAGGGGOCATTGCOGCTGCOCTOCCCGGATGGTCAGGTGTC
CACTGTGACAGTGTGTGCTGAGGGAGCTGGGGCCCCAAGTCTOCT
GCOCTGCTACTGTAAAAATGGGGCTTCATGCTOCCCTGATGATGGCATCT
GOGAGTGTGCAOCAGGCTTCOGAGGCAOCCACTTGTGAGGATCTGCTOC
OCTGGTTTTATGGGCATGGCTGCAGOCAGACATGCCCCAGTGGTTCA
CAGCAGGGGGGCTGOCACACATCAOCCGCTGTGTGACTGCTTGOCTG
GCTTCACAGGGGCOCTCTGCAATGAAGTGTGTCCAGTGGCAGATTTGGG
AAAAACTGTGCAGGAATTTGTACCTGCAOCCAAAGGAAOCTGTAAOCC
CATTGACAGATCTTGTGAGTGTACCCCGGTTGGATTGGCAGTGAAGTCT
CTCAOCCATGTCCOCTGCOCACTGGGGGOCACAACTGCATOCACAGTGC
AACTGOCATAATGGAGCTTTCTGCAGGOCCTAOGATGGGGAATGTAAATG
CACTOCTGGCTGGACAGGGCTCTACTGCACTCAGAGATGTCTCTAGGGT
TTTATGGAAAAGATTGTGCACTGATATGCAATGTCAAAAAGGAGCTGAC
TGGAOCCACATTTCTGGGCAGTGTACTTGOCCACTGGATTCTATGGGAOG
GCACTGTGAGCAGAAGTGOCTTCAGGAACATATGGCTATGGCTGTGOC
AGATATGTGATTGTCTGAACAACTOCAOCTGOGAOCACATCACTGGGAOC
TGTTACTGCAGCCCCGGATGGAAGGGAGOGAGATGTGATCAAGCTGGTGT
TATCATAGTTGGAATCTGAACAGCTTAAGCOGAAOCAGTACTGCTCTOC
CTGCTGATTCTACCAATGGGGGOCATTGCAGGCATCATCATTTCTTGT
CTAGTTGTCTCTOCTACTGGCATTGTTTATTATTATAGACACAGC

Fig. 7

>fthxe29c9R, 1578 bases, 5059 checksum.

NAGOOCAACAGGAATGTTCTATGAAAGTGAAOCTAACAGTGAGTGTGTGTT
OOCAAGGAGTATTCAGCAATAATGGGOGTCTNTOOCAAGGATOCATATGA
OCTOOCAAAGAACAGTCACATOOCTTGTCAATTATGACCTGCTGOCAGTOC
GAGACAGTTTCATCTCTOCTTAAAGCAAGAGGACAGTGGAGGTAGCAGCAGC
AACAGCAGCAGCAGCAGTGAATGACAOCAAGGAOOGCTTGGTAGOCACT
GGAAOOCCTTTCAGAACTGCTGTTTGGTTCTTCTOCATCTCAATTTTGC
CACTTTCATGTGAATGTTAGTCAATTCGGTGGGCAATTTTGGACATGAA
OCAGAAAGCTGAAAGCTGAGGCTGACACGGACTGTAGGTGCTTTTGTTC
AGGTGGATTTOGAAGGAGTTAGAGATGTGATTGOCATTGCTGTAGTTTT
AGAACTATAOCCGTGAAGCATGACTTATTGTAAGATGTTGGCTGAAAGCA
TGAACCTGCAGAACTOCTGGGAGAOGCAGGTTGCAGTGGACATTGGGAT
TGTTGCTTGAAAAATTAAAATTTGAATATTTTCTCTCTCATTTGCATCAT
ACAGCTCTAOCCTAGGATTGTACAGTTTACCATAAAATTTACTTCATGAAA
GTGGGAATCACTGAACATGTAGAAGACAAGGAACATATTGTTAACTOCTG
ATTCTTAACTTTATTCAACTGGACTCAGAATTGTAGGGATAATATGAATG
CAGGAGGAAACATTTCTGTGAGGOGGTATGACTGGACAGACTTTGAATATA
CTCTAAAAGTGGACAGAAAAATTTAOGAAATCTTAGATTTTGTTTAGAAT
GAGAAAATATACAATTAGAATTATTTAGAAATAGTAGGAAGTATTGCAG
AAGTCAATACACAAATGTGOCAGGCAGAGGTGGTTTTCTCTGTTTGACTC
TCAOCCAACCTTCAGATCTATGACATTATTCTGATCACTGGCTOCATCATA
CATATTCAOCCACTTGAGATTCATAACATATCAATAGTTATTTCAAAATA
TAGAAATGAAATAATTTTATTTTGAAGACTGGATGGAATGAGTGTGTGA
ATGATTGATAAAGGTTGTAAATTTTAAATGCAAGATGAOGCTTAOGTTCT
GTAAOCCATTAGTAATACATGCTGTAAATATAGAATTAGTGAACATTTTG
ATTAATCTTTCCCTAGAAGTGACTGAAATATTTTGTGCATATTTGAGAA
AGGGAACCTTTCCTTTTATTAATTGTCAATTTAGAGAACTATGCTTAAGC
TGGTCTTTTGCATTGCTAATGTGACATGTACCAACTTTTCATTAATTTG
TATTTCCATTTTAAATTCATATTCTATGTTTGTAGTGTTTGGATTGT
TAATGAAAAAATATTATATGTTCTGTTATTCCTTGTATTATTGOCATTTAT
CTTTTGTGTTGATAAAAAATGOGTTGTCTTTTCTTTTGGAGGGACAAGA
TGAAAAATATATAATTTGAATTGATTAAAATTGGTCTTACTAAAATAGTA
TAGTAAAAAAAAAAAAAGGGGGGGOOG

Fig. 8

TANGO 71/ADAMTS-1 Comparison (90% Protein Sequence Identity)

```

251 DQSMADFHSGSLKHYLLTLFSVAARFYKHPSIRNSISLVVVKILVITYEQ 300
      ||||:|
1 .....TRFILVTHDEQ 11

301 KGPEVTSNAALTILRFCSWQKQHNSPSDRDPEHYDTAILFTRQDLGSGT 350
      |||||:|
12 KGPEVTSNAALTILRFCSWQKQHNSPSDRDAEHYDTAILFTRQDLGSGT 61

351 CDTLGMAVGVTVCDPSRSCSVTEDDGLQAAFTTAHELGHVFMFPHDDAKH 400
      |||||:|
62 CDTLGMAVGVTVCDPSRSCSVTEDDGLQAAFTTAHELGHVFMFPHDDAKQ 111

401 CASLNGVSGDSHLMASMLSSLDHSQFWSPCSAYMVTSLFNGHGECIMDK 450
      |||||:|
112 CASLNGVSGDSHLMASMLSSLDHSQFWSPCSAYMVTSLFNGHGECIMDK 161

451 PQNPILKPSDLPGTLVDANRQCQFTFGEESEKHCPSDAASTCTLMCTGTSG 500
      |||||:|
162 PQNPILKPSDLPGTLVDANRQCQFTFGEDSEKHCPSDAASTCTLMCTGTSG 211

501 GLLVCQTKHFPMADGTSCEGEGWCVSGRVCNKTDMKHFPVHGSWGWG 550
      |||||:|
212 GLLVCQTKHFPMADGTSCEGEGWCVSGRVCNKTDMKHFPVHGSWGWG 261

551 FWGDCSRTCGGVQYTMRECDNPVFNKGKCYCEGRVRYRSCNLEDCPDN 600
      |||||:|
262 FWGDCSRTCGGVQYTMRECDNPVFNKGKCYCEGRVRYRSCNLEDCPDN 311

601 NGKTFREEQCEAHNEFSKASFGNEPTVEWTPKYAGVSPKDRCKLTCEANG 650
      |||||:|
312 NGKTFREEQCEAHNEFSKASFGSGPAVENIPKYAGVSPKDRCKLTCEANG 361

651 IGYFFVLQPKVVDGTPCSPDSTSVCVQGCVKAGCDRLIDSKKQFDRQGV 700
      |||||:|
362 IGYFFVLQPKVVDGTPCSPDSTSVCVQGCVKAGCDRLIDSKKQFDRQGV 411

701 CGGAGSTCKKMSGIVTSTRPGYHDIPTIPAGATNIEVKQRNQRGRNNGS 750
      |||||:|
412 CGGAGSTCKKMSGIVTSTRPGYHDIPTIPAGATNIEVKQRNQRGRNNGS 461

751 FLAIRAADGTYTLNGNFTLSTLEQDLTYAGTVLRYSGSSAALERIRSFSP 800
      |||||:|
462 FLAIRAADGTYTLNGNFTLSTLEQDLTYAGTVLRYSGSSAALERIRSFSP 511

801 LKEPLTIQVLNVGHALRPKIKFTYFMKQKTESFNAIPTFSEWIEENGEC 850
      |||||:|
512 LKEPLTIQVLNVGHALRPKIKFTYFMKQKTESFNAIPTFSEWIEENGEC 560

901 WSPCSKTCGRGYKKRMLKCVSHDGGVLSNESCDPLKKPKHYIDFCTLTC 950
      |||||:|
561 ...CSKTCGRGYKKRSLKCLSHDGGVLSNESCDPLKKPKHYIDFCTMAEC 607

951 S* 951
      |
608 S* 609

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Fig. 9

Comparison of TANGO 73 and RVP1

Percent Similarity: 68.750 Percent Identity: 48.077

c73pro x RVP1.pep

July 29, 1997 17:38 ...

```
1 MANAGLQLLGFI LAF LGWIGAIVSTALPQWRIYSYAGDNIVTAQAMY EGL 50
  . :|:: | ||. |||:.. ||: ||| ||: .: |.. |: ||| : |||
1 .MSMSLEITGTSLAVLGWLCTIVCCALPMWRVSAFIGSSIITAQITWEGL 49

51 WMSCVSQSTGQIQCKVFDSLNLSSTLQATRALMVVGILLGVIAIFVATV 100
  ||. || |||||: |||: ||||. |. . |||. |||: ||: |||: : : || |
50 WMNCV.QSTGQMCKMYDSLLALPQDLQAARALIVVSILLA AFGLLVALV 98

101 GMKCMKCLEDEDEVQKMRMAVIGGAIFLLAGLAILVATAWYGNRIVQEFYD 150
  | . | . |: : ||.. | : : : : |: ||||: : . ||: . | : | | : : ||:
99 GAQCTNCV.QDETAKAKITIVAGVLFLLAAVLTIVFVSW SANTIIRDFYN 147

151 PMTFVNARYEFGQALFTGWAAASLCLLGGALLCCSCPRKTTSYPTPRPYP 200
  |: . | . . : | : | : ||||. | ||||| ||||| : : . . ||. |.
148 PLVPEAQKREMG TGLYVGWAAAALQLLGGALLCCSCPPREKYAPTKILYS 197

201 KP...APSSGKDYV..... 211
  | :|: .|: .
198 APRSTGPGTGTGTAYDRKTTSERPGARTPHHHHYQPSMYPTRPACSLASE 247
```

Fig. 11

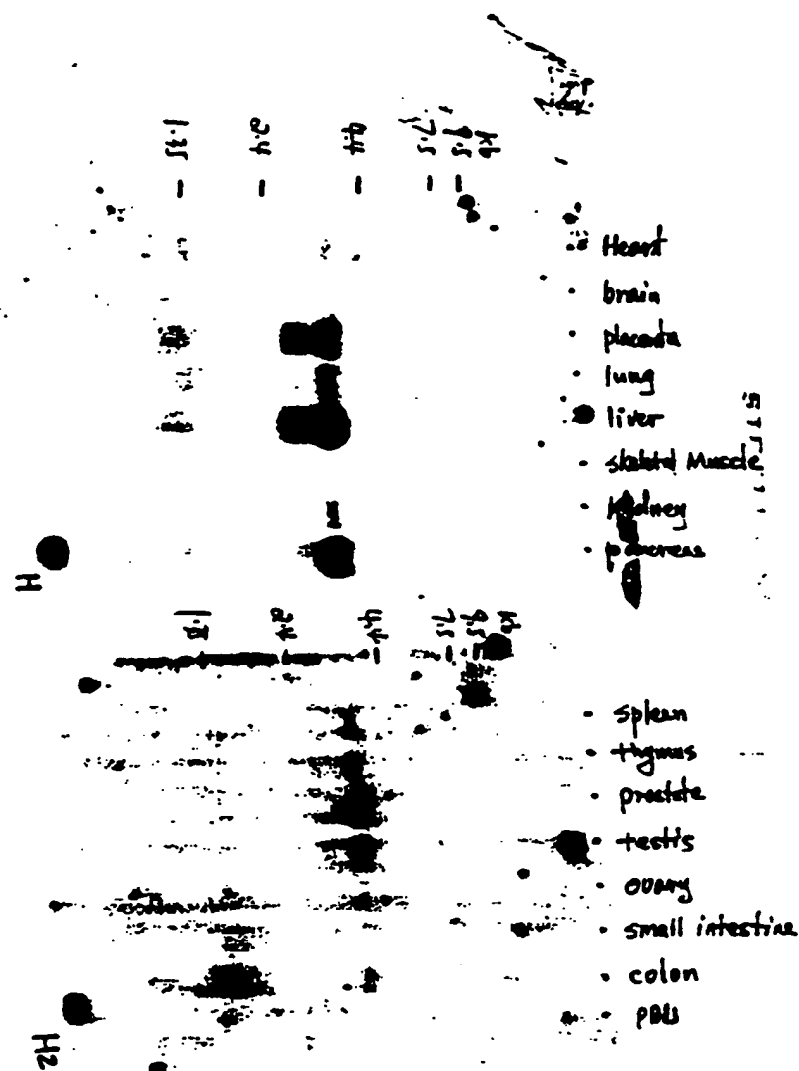
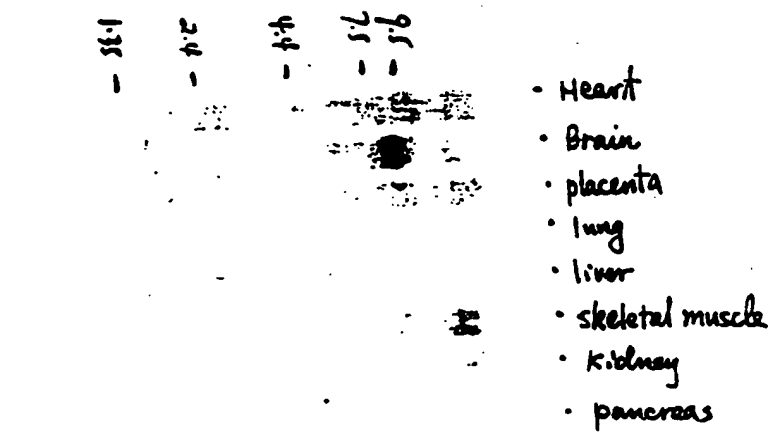
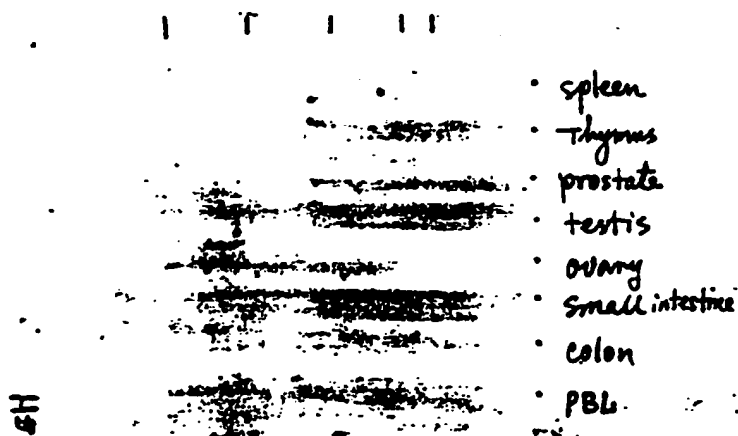


FIG. 12



H



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FIG. 13

FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16502

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/12, 15/16, 15/18, 15/63; C07K 14/46, 14/47, 14/475

US CL : 530/350; 435/69.1, 71.1, 71.2, 325, 471, 252.3, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/69.1, 71.1, 71.2, 325, 471, 252.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/01896 A1 (HUMAN GENOME SCIENCES, INC.) 25 January 1996, see entire document.	1-10, 12
A	BRADHAM et al. Connective Tissue Growth Factor: a Cysteine-rich Mitogen Secreted by Human Vascular Endothelial Cells Is Related to the SRC-induced Immediate Early Gene Product CEF-10. The Journal of Cell Biology. September 1991, Vol. 114, No. 6, pages 1285-1294, see entire document.	1-10, 12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

14 OCTOBER 1998

Date of mailing of the international search report

12 NOV 1998

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INTERNATIONAL SEARCH REPORT**International application No.**
PCT/US98/16502**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASON et al. Dorsal Midline Fate In Drosophila Embryos Requires Twisted Gastrulation, A gene Encoding A Secreted Protein Related To Human Connective Tissue Growth Factor. Gene & Development. 1994, Vol. 8, pages 1489-1501, see entire document.	1-10, 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/16502

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, MEDLINE, CAPLUS, EMBASE, BIOSIS

search terms: Tango-71, Tango-73, Tango-76, Tango-74, Tango-83, nucleic acid, DNA, polypeptide, protein, recombinant, cloning, production

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups invention which are so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must to paid.

Group I, Claims 1-10 and 12, drawn to Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, a vector, a host cell, and a method for producing Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides.

Group II, Claim 11, drawn to antibodies which bind Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides.

Group III, Claims 13-15, drawn to a method for detecting Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides in a sample.

Group IV, Claims 16-18, drawn to a method for detecting nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, & Tango-76 and Tango-83 polypeptides in a sample.

Group V, Claims 19-22, drawn to a method for identifying a compound which binds to Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, a vector, a host cell, and a method for producing Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides. Further pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.